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A new species of *Leptostroma* on *Pinus henryi* from ChinaLAN ZHANG¹, D.W. MINTER², QING LI¹, & YING-REN LIN^{3*}¹ School of Life Science & ³ School of Forestry & Landscape Architecture,

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ABSTRACT — A coelomycetous fungus found on dead fallen secondary needles of *Pinus henryi* from Shennongjia forestry region of Hubei Province, China, is described, illustrated, and discussed. Habitat, morphology, and conidial development place it in the *Rhytismataceae*. It differs from other members of this family on the same substratum by having conidiomata with upper walls more than one cell thick, and is formally named as *Leptostroma magnum* sp. nov. Its conservation status is evaluated as Data Deficient. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

KEY WORDS — anamorph, anatomy

Introduction

During a study of microscopic fungi associated with trees in the Shennongjia forestry region (Hubei, China) in 2010, an interesting coelomycetous species was found on dead fallen needles of *Pinus henryi*. The fungus was collected again in repeat visits to the same site made in 2012 and 2013. It is described, illustrated, and discussed below.

Materials & methods

Specimens were collected from the leaf litter of *Pinus henryi* trees, which were more than 20 years old in 2010. All collections were made at the same location, at an altitude of 1320 m, in Shennongjia forestry region, Hubei, China, in July 2010, October 2012, and April 2013. Specimens were deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF). For microscopic examination, only needles with sporulating conidiomata were used. External features and arrangement of the conidiomata and associated zone lines were observed with a dissecting microscope at 10–50× magnification. Specimens were rehydrated in water for 10 minutes, and 10–15 µm thick vertical transverse sections of conidiomata were

made using a freezing microtome (YD-202, China) and mounted in 0.1% (w/v) cotton blue with lactic acid. Measurements and drawings of conidiogenous cells and conidia were made from squash mounts in 5% KOH, with more than 30 conidiogenous cells and conidia measured for each specimen. Colors of the various structures were observed in water. Illustrations of external shapes and internal structures of the conidiomata were prepared using the Panasonic XSJ-2 microscope drawing device. Comparisons with other similar species were based on protologue descriptions and illustrations, and, where possible, examination of type material.

Taxonomy

Leptostroma magnum Y.R. Lin & Lan Zhang, sp. nov.

FIGS 1, 2

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Differs from other species of the *Rhytismataceae* on dead fallen secondary needles of diploxylon pines by having subcuticular conidiomata with upper walls more than one cell thick.

TYPE: China, Hubei, Shennongjia forestry region, Guanmenshan, alt. 1320m, on dead needles of *Pinus henryi* Mast. (*Pinaceae*), 10 July 2010, Y.R. Lin, J.L. Chen & Q. Zheng 2455 (Holotype, AAUF 68563).

ETYMOLOGY: *magnum* (Latin = large), referring to the size of the conidiomata.

ZONE LINES black, thin, frequent, with the black fungal tissue penetrating the stele. CONIDIOMATA on both surfaces of the needle, though usually much more frequent on the abaxial side, scattered, sometimes confluent in groups of two or three (especially longitudinally), 350–740 × 110–240 µm, oblong or elliptical, rugged, with a clearly marked outline, shiny black, slightly raising the substratum surface, opening by at least four more or less circular ostioles and one to several irregular tears in the upper wall. IN VERTICAL TRANSVERSE SECTION located underneath the needle cuticle and above the epidermal cells which are largely intact and yellow-brown to brown, with some hypodermal cells invaded by dark grey-brown angular fungal hyphae 2.5–4.5 µm diam. and others strongly brown perhaps as a result of accumulated tannins; somewhat domed, 70–80 µm deep, with an upper wall 8.5–13 µm thick, composed of blackish-brown angular-aliform thick-walled cells below the needle cuticle, becoming slightly pale (yellow-brown) and thin-walled towards the edge and not connecting to the basal wall which is extremely poorly developed. SUBCONIDI GENOUS LAYER colourless, 6–9.5 µm thick, consisting of textura angularis composed of cells 2–3.5 µm diam. with rather thick walls; a similarly structured but grey-brown semicircular raised area 40–70 µm wide sometimes also present in the centre. CONIDIOPHORES not observed. CONIDI GENOUS CELLS colourless, thin-walled, smooth, either ampulliform or cylindrical and tapering towards the apex, 12.5–20 × 2.5–4 µm, usually proliferating sympodially, rarely percurrently. CONIDIA 7.5–11.5 × 0.9–1.3 µm, colourless,

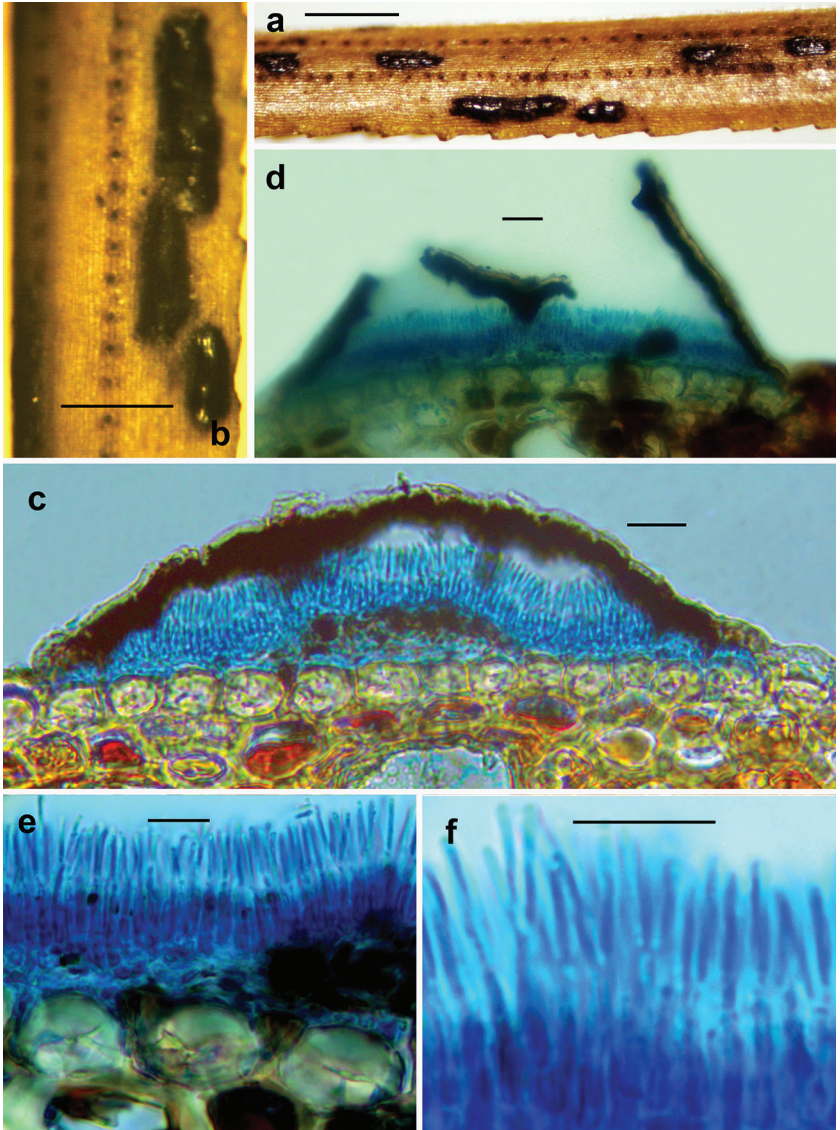


FIGURE 1. *Leptostroma magnum* (ex holotype AAUF 68563): a. Conidiomata on a needle. b. Detail of conidiomata. c. Conidioma in vertical section. d. Opening conidioma in vertical section. e. Conidiogenous cells, conidia, subconidiogenous layer and basal wall. f. Enlargement of conidia. Bars: a, b = 500 μ m; c, d = 20 μ m; e, f = 10 μ m.

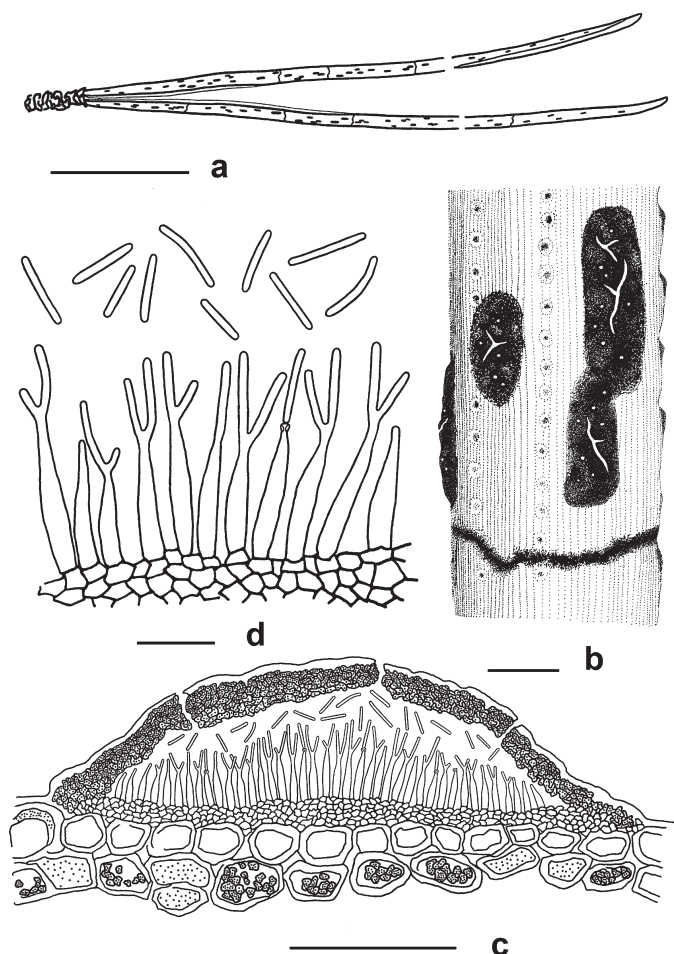


FIGURE 2. *Leptostroma magnum* (ex holotype AAUF 68563): a. Habit on needles. b. Detail of conidiomata and a zone line. c. Conidiomata in vertical section. d. Conidiogenous cells and conidia. Bars: a = 10 mm; b = 250 μ m; c = 50 μ m; d = 10 μ m.

aseptate, smooth, cylindrical, sometimes slightly curved, obtuse or round at the ends. TRICHOGYNES not observed. TELEOMORPH not observed.

ECOLOGY: The fungus was observed on 2–3-year old secondary needles, which had turned yellow-brown to straw yellow, died, and fallen into the litter.

ADDITIONAL SPECIMENS EXAMINED: On *Pinus henryi*: CHINA, HUBEI, Shennongjia forestry region, alt. 1320 m, October 2012, L. Zhang & S.J. Wang 2864 (AAUF 68972); April 2013, Y.R. Lin & Q. Li 2971 (AAUF 69079).

Discussion

From their habitat and general form, there can be little doubt that the zone lines and conidiomata in these collections are of a member of the ascomycete family *Rhytismataceae* Chevall. These fungi are seen on dead leaves, twigs, and sometimes other woody material of a wide range of plants. Some are plurivorous. Others are associated with only one or a few closely related species or genera of plants. Many exist as symptomless endobionts in living plant tissues. Some may be mutualistic. Others can be parasitic. A few are pathogens. Fruitbodies are only seen after death of colonized plant tissue. Zone lines defining the extent of individual colonies occur in many species. In some only a teleomorph is observed. In many, perhaps most, an anamorph precedes and accompanies the teleomorph. In a few an anamorph is produced but no teleomorph. Species of this family occurring on pines are generally not found on other plants.

The biology of this fungus poses interesting questions. Why is it only known as an anamorph? Is it possible that the teleomorph has simply not yet been found? In many of the *Rhytismataceae* on pine needles, the anamorph is produced before the teleomorph and, at the correct time of year, the anamorph can be seen but not the teleomorph. The time in this condition is generally rather short. Ascomatal initials usually appear within a month of the first conidiomata appearing (Minter 1977). The three collections of the current species were made at very different times of year, and none had evidence of a teleomorph, so this may be a species with no teleomorph. That would have significant implications for the fungus.

In general, for pine needle inhabiting members of the *Rhytismataceae*, ascomata on dead needles release ascospores, which disperse and colonize young living needles. The fungus then lives there as a parasite or symptomless endobiont until death of the colonized tissues. Then new conidiomata and/or ascomata form, completing the life cycle. Most anamorphs of the *Rhytismataceae* are thought to have a spermatial function (Jones 1935): they are there for sexual reproduction, not dispersal. The conidiomata, conidiogenous cells, and conidia of the present fungus look very similar to many of those anamorphs. If this fungus does not produce ascomata (the usual dispersal state in the *Rhytismataceae*), then what function do these conidiomata have, and how does the fungus colonize new substrata? The search for answers only raises further questions. Does this fungus only occur on *P. henryi*, or is it also on other pines nearby? If so, perhaps there is some reason why the fungus only produces conidiomata on *P. henryi* (for example some interaction within the needle may suppress teleomorph formation), and perhaps the fungus produces the teleomorph on those other pines. Alternatively, the fungus may occur only

on *P. henryi*, and may as a result have evolved different strategies to spread. One possible way might be if the fungus were systemic within the tree, i.e. not just in the needles but also in living twigs. At least one pine needle inhabiting member of the *Rhytismataceae*, *Elytroderma deformans* (Weir) Darker, has adopted that strategy (Lightle 1954). Such a fungus could at least theoretically spread as a symptomless endobiont of seeds. It is clear that the current fungus offers several interesting lines of investigation.

To establish its identity, the current fungus must be compared with other known species. Many members of the *Rhytismataceae* are known from pines, and more than 40 species have been observed on needles. Some fruit on dead portions of still attached living needles (species of the genera *Bifusella* Höhn., *Canavirgella* W. Merr. et al., *Davisomycella* Darker, *Elytroderma* Darker, *Lophodermella* Höhn., *Ploiderma* Darker, *Soleella* Darker). The present fungus, collected on dead fallen needles is very unlikely to be one of them.

Some of the litter-inhabiting species have no known anamorph (*Lophodermium canberrianum* W. Stahl ex Minter & Millar, *L. durilabrum* Darker, *L. erlangshanense* Y.G. Liu, *L. indianum* Suj. Singh & Minter, *L. macchi* Sokolski & Bérubé, *L. maximum* B.Z. He & D.Q. Yang, *L. puerense* C.L. Hou & M. Piepenbr., *L. sichuanense* D.X. Qiu & Y.G. Liu, *Meloderma sharmarum* P.F. Cannon & Minter). The present fungus, being known only as an anamorph, is also unlikely to be one of these species.

Although exceptions are known, there is a tendency for species of the *Rhytismataceae* on pine needles to occur on either haploxyton pines (i.e. species with one vascular bundle per leaf) or diploxyton pines (species with two vascular bundles per leaf), but not both (Minter 1977). As the current fungus was found on a diploxyton pine, it is likely to differ from species known on haploxyton pines (*Lophodermium anhuiense* Y.R. Lin, *L. confluens* Y.R. Lin et al., *L. ellipticum* Y.R. Lin, *L. himalayense* P.F. Cannon & Minter, *L. kumaunicum* Minter & M.P. Sharma, *L. mirabile* Y.R. Lin, *L. nitens* Darker, *L. orientale* Minter, *L. pini-bungeanae* Y.R. Lin, *L. pini-excelsae* S. Ahmad, *L. pini-sibiricae* C.L. Hou & S.Q. Liu).

Of the species known to have an anamorph and to occur on secondary needles of diploxyton pines, the following have conidiomata immersed beneath the epidermis: *Leptostroma pineae* (Bubák) Arx, *Lophodermium australe* Dearn., *L. baculiferum* Mayr, *L. conigenum* (Brunaud) Hilitzer, *L. guangxiense* Y.R. Lin, *L. iwatense* Sakuyama, *L. pinastri* (Schrad.) Chevall., *L. ravenelii* Minter, *L. seditiosum* Minter et al., *L. staleyi* Minter, *L. yuexiense* C.L. Hou et al. As the depth of embedding of conidiomata is a rather constant character at species level in the *Rhytismataceae* (Minter 1980), all of those species are also likely to be different from the current fungus, which has subcuticular conidiomata.

There remain four members of the *Rhytismataceae* known to produce subcuticular conidiomata on fallen secondary needles of diploxylon pines: *Lophodermium molitoris* Minter, *L. pini-mugonis* C.L. Hou & M. Piepenbr., *L. yanglingense* Z.M. Cao & C.M. Tian, *Meloderma desmazieresii* (Duby) Darker. All of them have conidiomata with either no upper wall, or with an upper wall only one cell thick. This is another rather constant species-level character in conidial states of the *Rhytismataceae*, and distinguishes those four species from the present fungus that produces conidiomata with upper walls several cells thick.

A few other names in the *Rhytismataceae* have been used for species inhabiting pine needles: *Cryocaligula hedgcockii* (Dearn.) Minter, *Leptostroma ahmadii* Petr., *L. austriacum* Oudem., *L. durissimum* Cooke, *L. decipiens* Petr., *L. pinorum* Sacc., *L. rostrupii* Minter, *L. strobicola* Hilitzer. These are all in genera traditionally considered as anamorphic, and are all known synonyms of species already discussed above. The present fungus thus seems different from all previously described members of the *Rhytismataceae* known from pines, because of its unique combination of characters: it inhabits dead fallen secondary needles of a diploxylon pine, producing subcuticular conidiomata where the upper wall is more than one cell thick.

Fallen needles of pine are known to be a hotspot substratum for sympatric evolution of the *Rhytismataceae* (Burnett 1983): they provide a remarkable range of habitats (the location of a needle on the tree can be significant, needles may be retained by a tree for different numbers of years, and they may die for different reasons). Subtle morphological variations can provide clues to the presence of different species, and molecular evidence is starting to show cryptic speciation where there is little or no morphological variation. There is also an increasing body of evidence that some members of the *Rhytismataceae* are highly specific in their plant associations. It is not surprising therefore to encounter new species in this environment.

Unfortunately, it is generally impossible to predict the teleomorphic genus to which conidiomata belong purely from appearance. Molecular techniques may help to resolve this dilemma. At least one species on pine needles recently found and known only as an anamorph (with subepidermal conidiomata) is being described in *Lophodermium* on the basis of molecular evidence (Koukol & Pusz, pers. comm.). Without molecular information, the only realistic option still remains to place the species in an anamorphic genus.

There are several anamorphic genera in the *Rhytismataceae*. Those accepted in the tenth edition of Ainsworth & Bisby's Dictionary of the Fungi (Kirk et al. 2008) are *Conostroma* Moesz, *Crandallia* Ellis & Sacc., *Cryocaligula* Minter, *Hysterodiscula* Petr., *Leptostroma* Fr., *Melasmia* Lév., and *Trybliodipycnis* Höhn.

Conidiogenous cells in all these genera are more or less compatible with the present species in shape, colour, size, and developmental characteristics. Ways in which the genera differ are reviewed in the following paragraph.

The type of *Conostroma* is the anamorph of *Colpoma quercinum* (Pers.) Wallr. It characteristically produces rather large multiloculate stromatic conidiomata in bark on twigs of *Quercus* (Sutton 1980). The type of *Crandallia* is the anamorph of *Bifusella acuminata* (Ellis & Everh.) Bonar & W.B. Cooke. It produces multiloculate conidiomata with an upper wall only one cell thick on dead leaves and dead portions of living stems of *Juncus* (Sutton 1980). The type of *Cryocaligula* is the anamorph of *Ploioderma hedgcockii* (Dearn.) Darker. It produces conspicuous unilocular conidiomata with upper walls several cells thick on dead portions of attached living needles of *Pinus*. It is also unusual in having large 1-septate characteristically snowshoe-shaped conidia with possibly a dispersal function, rather than the small aseptate conidia with a spermatial function found in most other anamorphs of the *Rhytismataceae* (Minter 1985). *Hysterodiscula* was originally described with two species, *H. empetri* Petr. and *H. kalmiae* Petr. Index of Fungi (1947) selected *H. empetri* as the generic type, and this choice was endorsed by Sutton (1977, 1980). Based on the information in Sutton (1980), conidiomata in this genus have an upper wall several cells thick, and large 0–1-septate cylindrical to clavate conidia each with a truncate base. The lectotype of *Leptostroma* is *L. scirpinum* Fr., the anamorph of *Hypohelion scirpinum* (DC.) P.R. Johnst. It produces uniloculate often coalescing conidiomata with an upper wall only one cell thick on dead stems usually of *Schoenoplectus lacustris* (L.) Palla (Sutton 1980). The type of *Melasmia* is the anamorph of *Rhytisma acerinum* (Pers.) Fr. It produces multiple conidiomata inside large black stromata on dead portions of living leaves and on dead attached or recently fallen leaves of *Acer*. The conidioma upper wall is only one cell thick. The type of *Tryblidiopycnis* is the anamorph of *Tryblidiopsis pinastri* (Pers.) P. Karst. It produces large complex often multilocular conidiomata with upper walls only one cell thick on twigs of *Picea*. Its conidia are long, thin and curved.

From this review, it is evident that the present species has a combination of characters not perfectly matched by any one of these genera: those producing conidiomata with thick upper walls are on woody material or have large septate conidia with a dispersal function; those on leaves produce conidiomata with thin upper walls and have conidia with a spermatial function. In external appearance, and in shape, size and development of conidiogenous cells and conidia, the present fungus looks like a species of *Leptostroma*. This is by far the largest anamorphic genus of the *Rhytismataceae*, and it has already been used for anamorphs of many members of the family inhabiting pine needles (Minter

1980; Sutton 1980; Cannon & Minter 1986; Lin et al. 2012). The only significant difference between the present fungus and *L. scirpinum*, the type of the genus, is that its conidiomatal upper walls are several cells thick.

Closer inspection of other pine needle inhabiting *Leptostroma* anamorphs shows that there is, in fact, considerable variation in conidiomatal upper walls. Where conidiomata are subepidermal, the upper wall is often paler, may be limited to an area round each ostiole (where present), or may even apparently be absent. Where conidiomata are subcuticular, the upper wall is often much blacker and much more extensive. The extent and pigmentation of conidiomatal upper walls may therefore be related, at least partly, to depth of embedding in the plant tissue, and that may reflect water availability and the need to protect against UV radiation (Sherwood 1981). In the present species, where the conidiomata are subcuticular, the upper wall is even more strongly developed. Until the biological implications of a thicker upper wall are better understood, upper wall thickness seems insufficient reason to establish a new genus, the fungus is therefore formally named below as a new species of *Leptostroma*.

Conservation status

Data Deficient. The species is poorly known and has been recorded from only one location (in a protected area which is part of the UNESCO Man & Biosphere network). If an obligate association with *P. henryi* were to be demonstrated, the conservation status of this fungus would automatically be at least Near Threatened, the current IUCN red list conservation status of its only known associated plant.

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